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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This proposed study will examine whether exposure to cadmium (Cd) from dietary or environmental sources increases the risk of breast cancer. We will examine this hypothesis using information collected from the California Teachers Study (CTS) cohort, a group of approximately 130,000 female school employees living in California followed for breast cancer since 1995. Information collected by questionnaire includes residential addresses, exposure to tobacco smoke, and food and beverage consumption. We will assess levels of dietary and environmental exposure by linking these collected data with available information on Cd residue levels in foods and beverages and environmental sources of Cd pollution near women's residences. In addition, we will estimate total Cd exposure by using existing urine samples provided by 304 women in the CTS to determine the relative contributions of dietary and environmental sources to the level of urinary Cd, which is considered a good measure of cumulative lifetime exposure. We will then evaluate whether dietary, environmental, and total exposure to Cd increase the risk of breast cancer.

To date, we have successfully completed the tasks scheduled for completion in the first year, including the acquisition and cleaning of datasets and the measurement of creatinine-adjusted concentrations of Cd in urine specimens collected from women participating in the validation sub-study. The mean concentration and standard deviation were 0.43 and 0.24 ug/g creatinine, respectively.

15. SUBJECT TERMS
Cadmium, diet, environment, breast cancer, biomarkers

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Table of Contents

INTRODUCTION

This proposed study will examine whether exposure to cadmium (Cd) from dietary or environmental sources increases the risk of breast cancer. We will examine this hypothesis using information collected from the California Teachers Study (CTS) cohort, a group of approximately 130,000 female school employees living in California followed for breast cancer since 1995. Information collected by questionnaire includes residential addresses, exposure to tobacco smoke, and food and beverage consumption. We will assess levels of dietary and environmental exposure by linking these collected data with available information on Cd residue levels in foods and beverages and environmental sources of Cd pollution near women's residences. In addition, we will estimate total Cd exposure by using existing urine samples provided by 304 women in the CTS to determine the relative contributions of dietary and environmental sources to the level of urinary Cd, which is considered a good measure of cumulative lifetime exposure. We will then evaluate whether dietary, environmental, and total exposure to Cd increase the risk of breast cancer.

BODY

In the first year of this project, we have completed the first two tasks listed in the Statement of Work. These tasks and their progress are documented in this section.

Task 1 (Months 1–6): Obtain IRB approvals. Obtain dietary residue and environmental pollutant databases related to cadmium (Cd) exposure.

a. Prepare and submit IRB applications for DOD and NCCC.
b. Obtain the following exposure databases: Total Diet Study (TDS; U.S. Food and Drug Administration), Hazardous Air Pollutants (HAPs; U.S. Environmental Protection Agency), Air Toxics Emissions Data System (ATEDS; California Air Resources Board), Annual Average Daily Traffic (AADT; California Department of Transportation).
c. Conduct processing and cleaning of exposure databases.
d. Develop exposure databases ready for linkage with California Teachers Study (CTS) data.

Progress: This task is complete. IRB approvals were obtained from both the Northern California Cancer Center (NCCC) and the USAMRMC in January and February 2008. We have also obtained, reviewed, and cleaned the databases that will be used to assess exposure to Cd from environmental and dietary sources. In addition, we also obtained the CTS dataset that includes cases of incident breast cancer (through 2007) as well as demographic and other risk factor information. Starting with the 133,479 women who completed the baseline questionnaire (in 1995-96), we excluded 13,076 women who did not have a geocodable residential address or live in California at baseline. We further excluded 6,144 women diagnosed with breast cancer prior to completing the baseline questionnaire, thus leaving a study population of 114,259 women for this project. In this study population, 4,419 women were diagnosed with breast cancer from baseline through 2007. The distributions of selected characteristics of this population are listed in Table 1.

Task 2 (Months 7–9): Measure urinary Cd concentrations from 24-hour samples provided by 304 validation sub-study participants and in repeat samples from 175 of the participants.

a. Ship 10 mL aliquots of all urine samples in bulk on dry ice to Pacific Toxicological Laboratories (PTL) in Chatsworth, CA.

b. Determine the Cd concentration using inductively coupled plasma mass spectrometry.

c. Obtain laboratory results from PTL including urinary Cd concentrations, method accuracy from urine standards, method precision from duplicates and detection limit from blanks.

Progress: This task is complete. We were able to retrieve complete initial urine specimens from 298 (98%) of the 304 validation sub-study participants as well as 140 (80%) of the 175 repeat samples that were collected 6–9 months after the initial sample. These samples were then shipped to PTL for the measurement of Cd and creatinine concentrations.

Urinary Cd concentrations (μg/L) were measured from 10 mL aliquots of the urine samples using inductively coupled plasma/mass spectrometry. The coefficient of variation was 5.4% for Cd standards over the weeklong analysis period. Creatinine levels (g/L) were determined using a modified-rate Jaffe method and were used to adjust Cd concentrations based on hydration to obtain creatinine-adjusted Cd levels (μg Cd/g creatinine). Using a detection limit of 0.1 μg/L, Cd was detectable in 98% of the samples. The median creatinine-adjusted Cd concentration was 0.40 μg/g creatinine

while the mean and standard deviation were 0.43 and 0.24 μg/g creatinine, respectively (Table 2). Previous studies have not reported median Cd concentrations, however the mean creatinine adjusted value of 0.43 μg/g observed in our study is very similar to the mean values from females in the Third NHANES (0.48 μg/g) and the mean level (0.44 μg/g) from a study of non-occupationally exposed women conducted in Wisconsin (Paschal et al., 2000; McElroy et al., 2007). Creatinine-adjusted Cd concentrations also increased with age in our study ($p<0.001$) from an average of 0.32 μg/g creatinine for women under 35 years of age to 0.58 μg/g creatinine in women over 72 years of age (Figure 1).

Among the 140 subjects with repeat urine samples collected 6–9 months later, the intra-class correlation coefficient for Cd concentration was 0.55 and for creatinine-adjusted Cd concentration it was 0.38 (Figure 2). This level of correlation agrees well with the results from previous studies ($r=0.4 – 0.6$) that have measured Cd in urine multiple times from the same subjects (Mason et al., 1998; Ikeda et al., 2005; Yamagami et al., 2008).

Task 3 (Months 7–15): Link the CTS food frequency questionnaire (FFQ) with the Total Diet Study database. Estimate dietary exposure to Cd for 111,526 subjects in the CTS cohort.

a. Estimate the mean Cd concentrations and standard errors for 103 food and beverage items listed in the CTS baseline FFQ, based on the assumption that contaminant levels in foods follow a log-normal distribution.
b. Add estimated Cd concentrations to the CTS nutrient database.
c. For each subject, estimate the average daily Cd intake (μg/day) based on the frequency and portion size of each food or beverage consumed as reported in the baseline FFQ.
d. For each subject, estimate total dietary Cd intake from all consumed foods and beverages as reported in the baseline FFQ.

Progress: This task is currently underway. Using data from the USDA Total Diet Study (TDS) (version 4.1, December 2007), we entered data on the Cd content of foods into our nutrient database. The mean values reported in the TDS were used. Of the 325 foods in the TDS with Cd values, approximately 200 overlapped with our nutrient database and were included. We are in the process of writing programs to convert the nutrient-specific data into individual-level exposure data based on the reported frequency and

portion size of each of the consumed foods or food groups included in our study's food-frequency questionnaires.

Task 4 (Months 7–15): Link CTS residential addresses with databases of environmental pollutants using a geographic information system (GIS). Estimate environmental exposures to Cd for all subjects in the CTS cohort.

a. Review accuracy and update residential address geocoding.
b. Link addresses with environmental pollutant databases (HAPs, ATEDS, AADT) using a GIS.
c. Estimate average exposure to Cd from environmental sources for 1995 - 2003 for all subjects in the CTS cohort.

Progress: This task is currently underway. We are currently conducting the linkage of residential addresses with the environmental datasets, which will be followed by the assessment of environmental exposures. We expect this task to be completed by December 2009.

One of the datasets we will be using for assessing environmental exposures is the 1995 ATEDS dataset, from which we identified 1,010 industrial Cd emission sources. Addresses for these sites were validated using USPS certified address standardization software (ZP4) and then batch geocoded using ArcGIS with NavStreets (NavTeq 1998Q4 version) as the primary street database. For verification purposes the addresses were also batch geocoded with another street database and an address point database (both from TeleAtlas). Only 60% of these addresses were successfully batch geocoded. This is remarkably low as previous experience indicates that typically over 90% of addresses batch geocode. Manual review of the addresses revealed that many of the entries in the address field were not valid street addresses. These can be categorized as:

1) Unable to geocode (e.g., oil field lease, offshore platform, berth, multiple or ambiguous address, or no address data at all);
2) Large facilities identified only by name and thus are difficult or impossible to identify the exact source of emissions within the facility (e.g., military base, shipyard, refinery, power plant, or mines);
3) Lacking a sufficient location description (e.g. near an intersection, at the end of a street, distance and direction from a street, Public Land Survey System coordinates, partial addresses, or P.O. boxes).

For categories 2 and 3, the available company name and location information was used to find a location or address using internet search tools and aerial imagery. This manual geocoding of ATEDS was undertaken by prioritizing the facilities with the highest levels of emissions, and then geocoding facilities with smaller emission levels down to a threshold of 0.1 pounds per year. Based on these data, Figure 3 maps the locations of facilities in California reporting Cd emissions $\geq$ 0.1 lbs per year and illustrates the cumulative distribution of these facilities by the total emitted lbs per year. Overall, 218 facilities emitted 0.1−1.0 lbs per year, 137 facilities emitted 1.0−10 lbs per year, and 65 emitted 10−3882 lbs per year.

Future Tasks: These tasks will be completed in the second and third years of the project, as indicated by the scheduled months:

Task 5 (Months 16–19)**:** Create the analytic dataset. Generate descriptive statistics.

a. Merge the datasets of dietary CD intake (entire CTS), environmental CD exposure (entire CTS), case-control status and relevant covariate information (entire CTS), and urinary Cd concentrations (validation sub-study) into a single analytic file.

b. Conduct preliminary descriptive analyses, evaluate variable distributions, and determine variable cut-points.

Task 6 (Months 20–24)**:** Evaluate the contribution from dietary and environmental sources to total Cd exposure based on urinary Cd concentrations.

a. Develop mixed-effects models.

b. Run these models with urinary Cd concentration as the dependent variable to calibrate exposures from dietary and environmental sources and other covariates in the validation sub-study population.

c. Conduct formal evaluation of effect modification with stratified models or models with interaction terms.

d. Evaluate model precision using iterative cross-validation.

Task 7 (Months 24–26)**:** Generate estimates of total Cd exposure for all subjects in the CTS cohort.

a. Apply β's estimated from mixed-effects models as weights for the dietary and environmental exposure estimates for all subjects in the CTS.

b. Estimate total Cd exposure for all CTS subjects.

Task 8 (Months 27–32)**:** Estimate the effects of total, dietary, and environmental exposure to Cd on breast cancer incidence in the CTS from 1996 to 2005.

a. Develop Cox proportional hazards models for estimating effects of exposure to Cd on breast cancer risk in the CTS.

b. Estimate hazard ratios for Cd exposure from specific sources and from all sources.

c. Conduct formal evaluation of effect modification.

Task 9 (Months 33–36)**:** Prepare final reports, finalize manuscripts and present findings.

a. Discuss and interpret study findings and their implications.

b. Prepare final reports.

c. Write manuscripts.

d. Present findings at scientific meetings.

KEY RESEARCH ACCOMPLISHMENTS

- Acquisition of environmental, dietary, and cohort datasets.
- Linkage of Cd concentrations in the USDA TDS dataset with the dietary database.
- Geocoding of Cd-emitting facilities.
- Measurement of creatinine-adjusted Cd concentrations in urine specimens collected from women participating in the validation sub-study.

REPORTABLE OUTCOMES

We have applied for a grant from the National Institutes of Health to examine the effects of dietary and environmental exposures to Cd on the risk of endometrial cancer. This application was motivated by our current study of Cd and breast cancer funded by USAMRMC and a recent report of an elevated risk of endometrial cancer associated with higher dietary intake of Cd among postmenopausal women enrolled in the Swedish Mammography Cohort (Akesson et al., 2008). This grant proposal is currently being reviewed.

CONCLUSION

We successfully completed the tasks scheduled for completion in the first year of this study. The completion of the assessment of dietary intake and environmental exposure in the second year of the study followed by analyses estimating the effects of these exposures on the risk of breast cancer will contribute to the growing body of evidence regarding the carcinogencity of Cd.

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SUPPORTING DATA

Table 1. Characteristics among the CTS participants with no prior history of breast cancer through 2007 and who resided in California at the time of the baseline questionnaire (n=114,259)

Table 2. Cd concentrations from initial urine specimens (n = 298)

Figure 1. Mean creatinine-adjusted Cd concentrations from initial urine specimens, by age at time of specimen collection (n = 298)

Figure 2. Scatterplot of urinary creatinine-adjusted Cd concentrations from initial and repeat samples (n = 140)

Figure 3. Map and cumulative distribution of facilities in California with reported Cd emissions $\geq$ 0.1 lbs/year, by amount of emissions, Air Toxics Emissions Data System (1995)

Table 1. Characteristics among the CTS participants with no prior history of breast cancer through 2007 and who resided in California at the time of the baseline questionnaire (n=114,259)

Characteristic	Case (n=4,419)	Non-case (n=109,840)	Total (n=114,259)
	%	%	%
Race			
White	89.0	86.1	86.2
Black	2.6	2.7	2.7
Hispanic	2.7	4.4	4.4
Asian/PI	3.3	3.7	3.7
Other	2.4	3.1	3.0
Age (years) (mean (SD))	58.2 (11.8)	52.6 (14.5)	52.8 (14.5)
Family history of BC			
Yes	17.5	11.5	11.7
No	79.0	84.7	84.5
Unknown	3.5	3.8	3.8
Age at menarche (years)			
≤11	23.4	22.1	22.1
12-13	55.4	55.9	55.9
≥14	19.8	20.4	20.4
Unknown	1.4	1.6	1.6
Age at first full-term pregnancy (years)			
Nulliparous	22.7	26.5	26.3
≤24	27.8	25.5	25.6
24-29	30.8	29.1	29.2
≥30	16.9	16.9	16.9
Unknown	1.8	2.0	2.0
Breast feeding history (months)			
Nulliparous	17.8	20.5	20.4
Pregnant without a live birth	4.8	5.9	5.8
None	19.7	15.9	16.1
< 6	18.3	17.6	17.5
6-11	14.0	13.5	13.5
≥12	23.2	24.3	24.3
Unknown	2.2	2.5	2.4

Table 1 (continued).

Physical activity (hours/week)			
0.00-0.50	36.2	29.8	30.0
0.51-2.00	31.7	31.8	31.8
2.01-3.50	15.0	17.5	17.4
3.51-5.00	8.5	9.5	9.5
>5.00	7.6	10.6	10.5
Unknown	1.0	0.7	0.8
Alcohol consumption (g/day)			
None	29.1	32.1	32.0
<20	55.0	54.8	54.8
≥20	11.0	7.6	7.7
Unknown	4.9	5.5	5.5
BMI (kg/m^2)			
16.0-25.7	60.3	63.9	63.7
25.8-32.2	27.5	23.5	23.7
32.3-54.8	7.8	8.2	8.2
Unknown/outlier	4.4	4.4	4.4
Menopausal status & HT use			
Pre-menopausal	22.2	41.2	40.5
Peri/post-menopausal & no HT use	12.4	11.8	11.8
Peri/post-menopausal & past HT use	7.5	6.8	6.9
Peri/post-menopausal & current HT use	42.5	26.8	27.4
Other/unknown	15.4	13.4	13.4
Smoking status			
Never	57.9	66.5	66.2
Former	34.7	27.9	28.1
Current	6.5	5.0	5.1
Unknown	0.9	0.6	0.6
Among former/current smokers:			
Total pack-years of smoking (mean (SD))	17.9 (18.7)	15.0 (17.6)	15.1 (17.6)
Average number of cigarettes smoked per day (mean (SD))	13.7 (10.5)	12.5 (10.2)	12.6 (10.3)
Among former smokers:			
Total years since quit smoking (mean (SD))	20.4 (11.3)	19.3 (11.5)	19.4 (11.5)

Table 2. Cd concentrations from initial urine specimens (n = 298)

	Cd (μg/L)	Creatinine (g/L)	Creatinine-adjusted Cd (μg/g)
25th Percentile	0.20	0.54	0.30
Median	0.30	0.70	0.40
75th Percentile	0.40	0.96	0.50
Maximum	2.0	2.5	1.5
Mean (SD)	0.33 (0.24)	0.79 (0.36)	0.43 (0.24)

Figure 1. Mean creatinine-adjusted Cd concentrations from initial urine specimens, by age at time of specimen collection (n = 298)

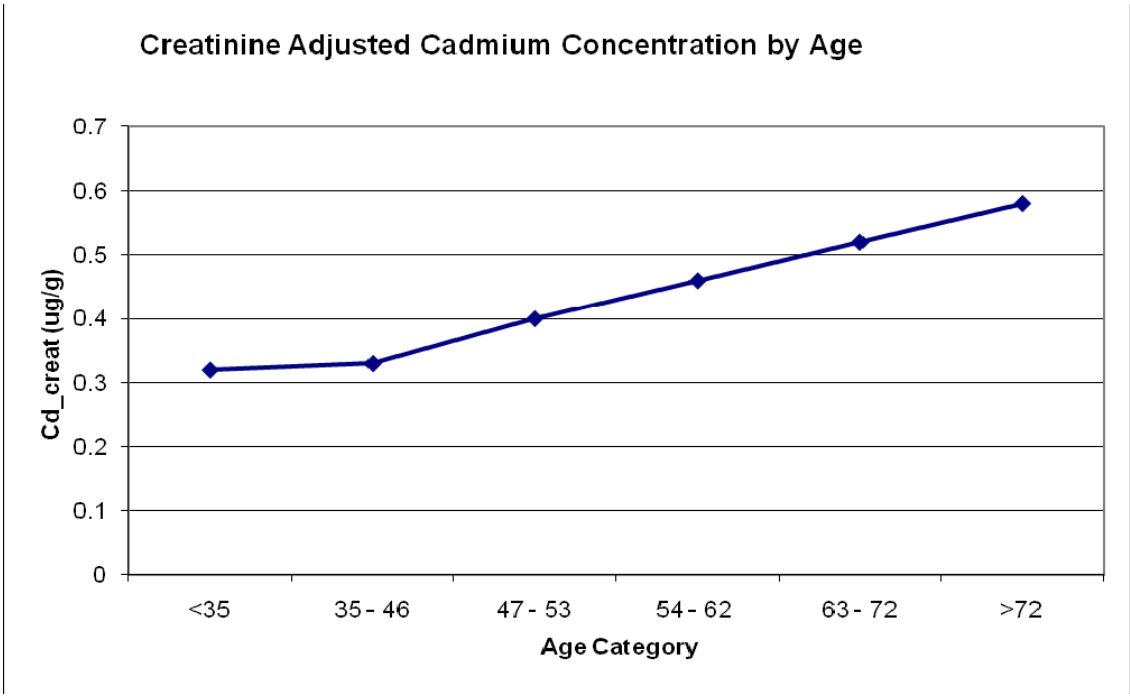

Figure 2. Scatterplot of urinary creatinine-adjusted Cd concentrations from initial and repeat samples (n = 140)

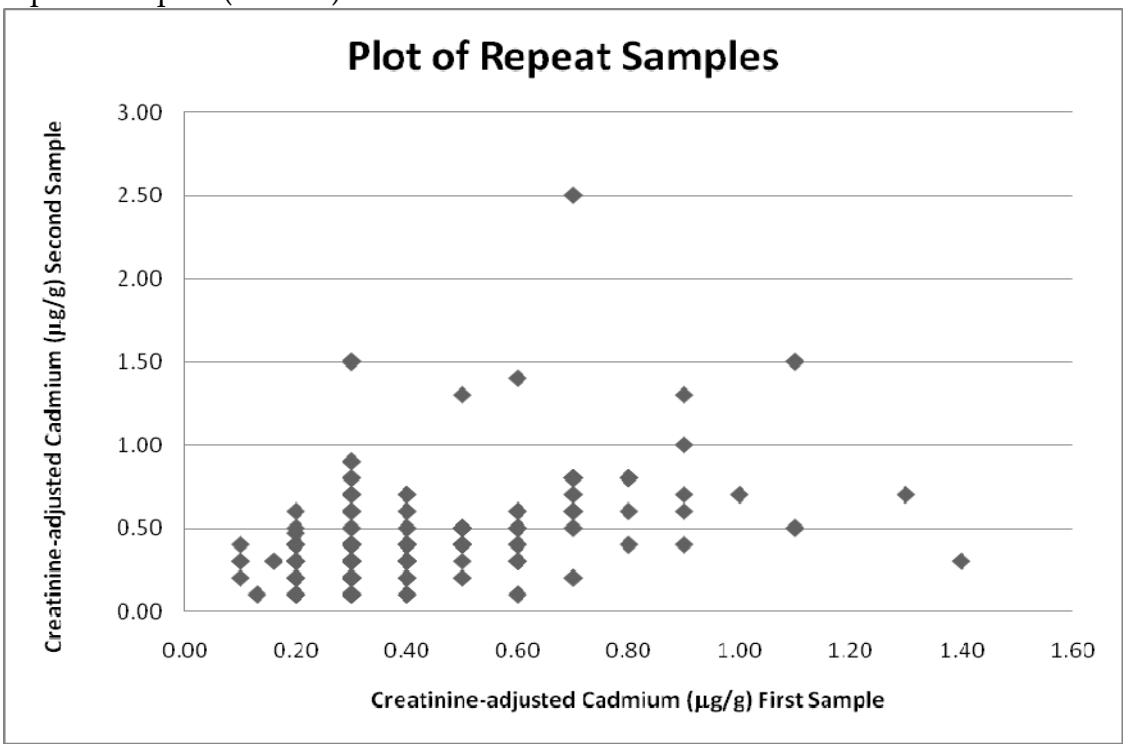

Figure 3. Map and cumulative distribution of facilities in California with reported Cd emissions ≥ 0.1 lbs/year, by amount of emissions, Air Toxics Emissions Data System (1995)